1. SCOPE: To provide guidelines for the typing and interpretation of short tandem repeat (STR) results generated using the Y-filer™ kit.

2. DNA Quantitation Interpretation Guidelines for ABI Quantifiler Y

2.1. Background:

2.1.1. The Quantifiler Y uses a real-time PCR reaction with fluorescent dye chemistry to estimate the quantity of amplifiable male DNA in each test sample.

2.1.2. DNA quantitation results are interpreted by using the analysis settings of the ABI 7000 instrument (or equivalent), the slope of the standard curve of the DNA standards, and the R^2 value of the DNA standards.

2.2. Slope:

This value indicates the efficiency of the PCR reaction of the quantification assay. A slope value of -3.32 indicates 100% amplification efficiency. The range of values that is acceptable to deem the assay results as valid is -3.0 and -3.6. If the value falls outside of this range, then one point of the slope may be dropped to account for pipetting variations. If a point is dropped, then both the original and adjusted slopes must be included in the case notes.

2.3. R^2 value:

This value measures the closeness of fit between the standard curve and the Ct values of the DNA standard used in the quantification assay. A value of 1.00 indicates a perfect fit between the standard curve line and the DNA standard data points. In order for the results from a given assay to be valid, the R^2 value must be greater than or equal to 0.98.

2.4. Internal Positive Control:

Each reaction in the Human Quantifiler Kit contains an Internal Positive Control (IPC) to help determine whether a sample is a true negative or whether there is inhibition occurring in the PCR reaction. The normal Ct values for the IPC should range from 20 to 30.

2.5. Interpreting IPC amplification results:

Quantifiler (FAM Dye)	IPC (VIC Dye)	Interpretation
No amplification	Amplification	True negative
No amplification	No amplification	Invalid result
Amplification (low Ct and high delta Rn)	No amplification	Disregard IPC result
Amplification (high Ct and low delta Rn)	No amplification	Partial PCR inhibition

2.6. No Template Control (NTC) and Extraction Controls

2.6.1. NTC: For each set of samples analyzed, a ANo Template Control@ (NTC) must be run to demonstrate that there was no contamination during the setup of the assay. Due to the sensitivity of the real-time PCR method, extremely low levels of DNA (levels that do not effect downstream applications such as STR analysis) may be detected. Therefore, if low level DNA with a Ct value of 36 or greater is observed in the NTC, it is still considered to be a negative sample and the results of the assay are still valid. However, if the Ct value of a NTC is less than 36, the NTC sample **may** be contaminated and the entire assay **may** be repeated. However, if the other negative controls show Ct values greater than 36, the analyst may proceed with the amplification of the samples.

2.6.2. Negative Extraction Controls: If the Ct value of a negative extraction control is less than 36, the analyst may either re-extract the set or continue processing the samples, realizing that activity may be observed in those controls in the post amplification product.

3. Y-STR Interpretation

3.1. Introduction: The interpretation of results in casework is a matter of professional judgment and expertise. These criteria are based on our validation studies, literature, and over 15 years of forensic DNA casework experience by this laboratory. However, it is not possible to address every situation with a pre-set rule. It is the responsibility of the analyst to use these guidelines in conjunction with their training and experience to provide a solid scientific interpretation of the results.

3.2. Preliminary Evaluation of Data

3.2.1. General

3.2.1.1. The Peak/Height cut off in the GeneMapper$_{ID}$ software will be set at 75 RFU for Y-STR Testing.

3.2.1.2. In general, activity below threshold should not be used for qualitative or quantitative data interpretation (e.g. evidence for a mixture within a given sample, allele drop out).

3.2.1.3. A general rule is that the Peak/Height to Noise (background) ratio should be 3:1. In other words, the Peak/Height should be at least 3 times greater than the average background for a peak to be called.

3.2.2. Positive Amplification Control: The Positive Amplification Control must have peaks that are in the proper location relative to the allelic markers. If these characteristic peaks are not in their correct position or are not present (too weak to interpret), that particular locus must be considered inconclusive for all samples and must be successfully re-injected, re-run, or re-amplified and analyzed before that locus may be used for analysis.

3.2.3. Negative Controls:

3.2.3.1. If any peaks are detected in the amplification negative control or the reagent control samples, then contamination *may* have occurred and the samples shall not be interpreted at the locus or loci in question. If possible, the sample(s) associated with the negative controls will be re-analyzed (i.e. re-injected, re-amplified, or re-extracted).

3.2.3.2. Artifacts observed in the negative samples shall not cause those samples to be re-analyzed. Those artifacts shall be documented in the notes.

3.2.4. Positive Extraction Control: The known bloodstain from MJB is used primarily as an extraction control and the samples may be interpreted if it fails to amplify at all or any loci.

3.2.5. Allelic Ladders: The peaks must be equal to or greater than 75 RFU. If regions of the ladder samples are not present, the specimen peaks shall not be interpreted in these regions. The size standards must be present and correctly called. The 250 bp size standard in the samples and controls shall

not differ ∀ 0.5 base pair from the ladder.

3.3. Samples

3.3.1. Visually inspect the known and questioned samples. Assess the quality of the peaks including RFU values and if artifacts are present. The peaks must be equal to or greater than 75 RFU for alleles to be called.

3.3.2. If a sample has more than one peak at a locus (except for DYS385), then the results *may* indicate a mixture. NOTE: If more than one peak is observed at a locus other than DYS385, then there may not be a mixture; the individual contributor may have a repeated sequence. Both sample and standard should express the same pattern in order to call a MATCH.

3.3.3. Failure of any loci to amplify for a multiplex STR system will not preclude the analysts from reporting those loci that are present, even if only one locus amplifies.

3.3.4. Samples that are overblown may need to be reinjected at a shorter injection time (if possible) or re-amplified using a lower amount of DNA template, depending on the overall quality of the electropherogram.

3.3.5. It is permissible to combine results from different injections (including dilutions) of the same sample when determining a final DNA profile.

3.4. Artifacts: The PCR process produces artifacts that are known and well characterized. The following artifacts should be appropriately labeled on the electropherograms in the case notes.

3.4.1. Stutter

3.4.1.1. The STR results should not be considered to be inconclusive if stutter peaks are present in single source samples. Care must be taken when interpreting samples where mixtures are present in regards to peaks in the stutter position. Peaks approximately one repeat smaller or larger than a true allele can be considered stutter peaks. The majority of the stutter associated with YSTR loci follows the same n-4 tendencies as autosomal STRs. However, note the following differences.

3.4.1.1.1. Stutter artifacts of both n-4 and n-2 occur with the locus DYS19. Deletion of the n-2 peak, typically labeled as "OL," should be called off and annotated as "Stutter" or "n-2".

3.4.1.1.2. The locus DYS392 may exhibit n+3 stutter in addition to n-3 stutter.

3.4.1.1.3. The locus DYS438 may exhibit n-5 stutter due to the pentanucleotide repeat.

3.4.1.1.4. The locus DYS448 may exhibit n-6 stutter due to the hexanucleotide repeat.

3.4.1.2. Stutter peaks are considered PCR artifacts and are not considered part of the DNA profile, but their presence should be noted. The GeneMapper$_{ID}$ software contains designated cutoff for peaks in stutter positions and will be used for designating stutter. Based upon analyst discretion, a minor peak in the stutter position that is called by the GeneMapper$_{ID}$ software may be disregarded as stutter if the peak in question is 1) not in a mixed sample and 2) when compared to the predominant sister allele, it is close to the percent stutter cutoff for that particular locus. If a mixture is observed, then great care must be used in interpreting weaker peaks in the stutter position.

3.4.1.3. The following table gives the maximum % of stutter generally observed for each locus. Peaks in a stutter position with a higher percentage may be considered true alleles.

Locus	Repeat size	% Stutter Cut Off	
DYS456	4	13.67	
DYS389I	4	7.81	
DYS390	4	12.45	
DYS389II	4	16.13	
DYS458	4	13.08	
DYS19	4	11.12	9.53 (n-2)
DYS385	4	15.38	
DYS393	4	12.01	
DYS391	4	8.66	
DYS439	4	8.72	
DYS635	4	13.04	
DYS392	3	15.03	8.07 (n+3)
YGATAH4	4	10.68	
DYS437	4	7.31	
DYS438	5	3.96	
DYS448	6	4.22	

3.4.2. Pull up: Generally, pull-up can be noted when all the alleles are overlapped using the software and the Apull-up@ is observed as a relatively small peak located directly under the larger peak of another color channel. Analysts should be aware of this phenomenon and use the computer software to aid them in discerning actual alleles from pull-up.

3.4.3. Unincorporated Dye: Analyst should not call Adye-blobs@ as an actual allele. ADye-blobs@ should be noted in the case file but not considered for interpretation.

3.4.4. Assigning Values to Microvariants and Off Ladder Alleles

3.4.4.1. The GeneMapperJID determines the base pair sizes of all peaks. The analyst may add the allele call by determining the correct allele size typing in the correct allele designation based on the base pair size.

3.4.4.2. Variant alleles that vary by less than the consensus repeat unit

will be designated as an integer of that variation (for example DYS438 8.2 allele).

3.4.4.3. If an allele falls above the largest value or below the smallest value of the allelic ladder for a locus, the allele will be designated as either greater than (>) or less than (<) their respective allelic ladder.

3.4.4.4. Microvariants must be documented in the case notes by printing either the Sizing Table or the Allele Plots in Genemapper ID that show the base pair size of the Microvariants and corresponding Ladder alleles.

3.4.5. Shoulder and Tail: Shoulders and tails will not prevent the analyst from assigning the specific peak an allelic value.

3.5. Comparison of Profiles

3.5.1. The comparison and interpretation of DNA profiles is primarily a qualitative judgment based on careful review by a qualified analyst, utilizing all information pertinent to the tests undertaken.

3.5.2. Matches and non-matches are determined by careful, objective qualitative and quantitative evaluation of the entire profile produced by the various loci tested. It is scientifically acceptable for a match or non-match to be determined for a case when one or more of the loci yield inconclusive results. A match will be based only on loci which yield conclusive results.

3.5.3. Match: DNA profiles are considered to match if their patterns are the same after taking into consideration the properties of the substrate tested and limitations of the specific techniques used.

3.5.4. Non-Match: Assuming a single source or predominant profile is obtained from a forensic sample, two DNA profiles are considered to be a non-match if there is a difference of even one allele after taking into consideration the circumstances of collection and preparation of samples and knowledge of the properties of the substrate tested and limitations of the specific techniques used.

3.5.5. Inconclusive results for an entire sample or for minor alleles in a mixture may be obtained. Inconclusive results are usually the result of an insufficient quantity of DNA or complete degradation of DNA present in a sample or a complex mixture. Inconclusive results may result from, but are

not limited to, the following causes:

3.5.5.1. Insufficient amounts of DNA for that locus in one or more of the samples tested.

3.5.5.2. Degradation of one or more of the peaks in any sample tested.

3.5.5.3. Preferential amplification due to great differences in amounts of DNA present in a sample from multiple contributors.

3.5.5.4. Inhibition: Inhibition is total or partial suppression of the PCR process that would result in partial or no DNA profile being obtained.

3.5.5.5. A complex mixture.

3.6. Conclusions

3.6.1. Single Contributor

Included below is a list of statements that may be used when reporting the results of Y-STR DNA analysis and when a single source profile is observed. This is a general list and may not include all possible scenarios. The statements should be used if the scenario fits. However, other statements may have to be used to fit scenarios not included below. Any additional statement should be consistent with the DNA results and conservative in nature.

3.6.1.1. Match (Complete Profile)

If the DNA profile from a known sample matches the DNA profile from a single contributor, then the results will be reported as follows:

*AThe male DNA profile obtained from (Item) **MATCHED** the DNA profile obtained from the bloodstain of the , (Item) and **DID NOT MATCH** the male DNA profile obtained from the bloodstain of the , (Item).”*

3.6.1.2. Match (Partial Profile of a Sole Sample)

If a sole forensic sample exhibits a partial profile and matches at those loci where data were obtained, then the following statement may be used:

*AA partial male DNA profile was obtained from (Item). The partial DNA profile **MATCHED** the DNA profile obtained from the bloodstain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the bloodstain of the , (Item). Results were obtained at the following genetic markers: (choose appropriate loci: DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19, DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448.*

Or:

AA partial male DNA profile was obtained from (Item). The partial male DNA profile was consistent with the DNA profile obtained from the bloodstain of the , (Item). The DNA profile of the , (Item) was not observed in the partial DNA profile. Results were obtained at the following genetic markers: (choose appropriate loci*: DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19, DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448.*)

3.6.1.3. Match (Partial Profile of Additional Samples)

If one or more samples Match at all loci and subsequent samples exhibit a partial profile but Match at those loci where data were obtained, then the following statement may be used:

*AA partial male DNA profile was obtained from (Item). The partial DNA profile obtained from (Item) **ALSO MATCHED** the DNA profile obtained from the bloodstain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the bloodstain of the , (Item). Results were obtained at the following genetic markers:* (choose appropriate loci*: DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19, DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448.*)

Or:

AA partial male DNA profile was obtained from (Item). The partial DNA profile was consistent with the DNA profile obtained from the bloodstain of the , (Item). Results were obtained at the following genetic markers: (choose appropriate loci*: DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19,*

DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448.)

Note: Population Frequency Data may be calculated in this situation if necessary. If Population Frequency Data are not calculated, the following phrase should be added*:*

"No Y-STR Database search was performed for this Item."

3.6.1.4. Non-Match

If the DNA profile from a known sample does not match the DNA profile from a single contributor, then the results will be interpreted as follows:

AAn unknown male DNA profile was obtained from (Item) that ***DID NOT MATCH*** *the DNA profile obtained from the bloodstain of the victim, (Item) or the DNA profile obtained from the bloodstain of the suspect, (Item).*

Or

AA partial male DNA profile was obtained from (Item) at __ of the 16 genetic markers tested that **DID NOT MATCH** *the bloodstain of the victim, (Item) or the DNA profile obtained from the bloodstain of the suspect, (Item). Results were obtained at the following genetic markers:* (choose appropriate loci *DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19, DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448.)*

3.6.2. Mixtures with a Major/Minor Contributor

3.6.2.1. A sample may be considered to consist of a mixture of major and minor contributors if there is a distinct contrast in RFU values (approximately > 50%). In cases where there is clearly a major and minor contributor (higher peaks indicating a major or predominant profile and lower peaks indicating a minor profile), a predominant DNA profile may be attributed as coming from a known contributor in the case of a Match.

3.6.2.2. If a predominant profile is present, the minor alleles shall be designated on the Allele Call sheets by placing parenthesis

around the minor allele(s). All loci must be evaluated in making this determination.

3.6.2.3. If there is evidence of a mixture of only two profiles in the mixture and the predominant profile shares the same alleles at a locus as the minor contributor, then predominance can be called at that locus.

3.6.2.4. It is acceptable for analysts to define assumptions based on the data observed in the notes. For example, the analyst may preface a mixture statement with AAssuming two contributors,...@ or AAssuming multiple contributor,....@.

3.6.2.5. Included below is a list of statements that may be used when reporting the results of DNA analysis and a mixed profile is observed. This is a general list and may not include all possible scenarios. The statements should be used if the scenario fits. However, other statements may have to be used to fit scenarios not included below. Any additional statement should be consistent with the DNA results and conservative in nature.

3.6.2.5.1. Mixture with Predominant Profile - Where multiple contributors are possible (e.g. suspect and consenting partner), all peaks present in the questioned sample can be accounted for by the standards and a clearly predominant profile is observed, the laboratory report may state:

A The male DNA profile obtained from (Item) is CONSISTENT WITH A MIXTURE. The predominant profile MATCHED the DNA profile obtained from (Item).”

If a known standard (i.e. consenting partner) cannot be excluded as a contributor to the mixture, the laboratory report may additionally state:

APerson=s name, (Item #) cannot be excluded *as a contributor to the mixture.@*

If a known standard can be excluded as a contributor to the mixture, the laboratory report may additionally state:

ÂPerson=s name, (Item #) was excluded as a contributor in the mixture.@

3.6.2.5.2. Mixture: Partial/Incomplete Profile with a major/minor contributor

In cases where an incomplete match or mixture is obtained (i.e. one or more loci cannot be used for comparison purposes), the analyst will specify the number of matching loci and loci that are conclusive.

ÂThe partial male DNA profile obtained from (Item) is indicative of originating from more than one contributor and is CONSISTENT WITH A MIXTURE. The predominant profile MATCHED the DNA profile obtained from (Item). The weaker profile is CONSISTENT WITH the DNA profile obtained from (Item). The match for Item was made for (choose appropriate loci *DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19, DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448*).*"*

Or:

ÂA partial male DNA profile was obtained from (Item). The partial DNA profile was consistent with the DNA profile obtained from the bloodstain of the , (Item). Results were obtained at the following genetic markers: (choose appropriate loci *DYS456, DYS458, DYS393, DYS392, YGATAH4, DYS389I, DYS19, DYS391, DYS437, DYS390, DYS385, DYS439, DYS438, DYS389II, DYS635 and DYS448*)*."*

3.6.3. Mixture with no major/minor contributor

3.6.3.1. If the profile is an unresolved mixture, no inclusion interpretations will be made due to the unavailability of statistical formulas for haplotype mixtures. It may be possible to exclude an individual from an unresolved mixture.

In this case the report should state:

"No conclusion can be made as to the donors of the male DNA profile obtained from _____(Item #)."

3.6.3.2. In instances where a contributor is known or expected to be present (intimate samples), the assignment of the known contributor's alleles may allow for the determination of the remaining profile. The remaining profile may be used for the determination of a match and subsequent database search to generate statistics. If this is a partial profile, the loci used for the database search must be listed in the report.

In this case the report should state:

"The male DNA profile obtained from (Item) is CONSISTENT WITH A MIXTURE. The DNA profile that did not originate from _____ (Item) MATCHED the DNA profile from the ___ (Item)."

3.6.4. Inconclusive Results

In the case where an analyst does not have sufficient data to reach a conclusion, then the report should state:

"No conclusive male DNA profile was obtained from _____(Item #)."

3.6.5. No DNA Results

In the case where no profile is obtained from an item, then the report should state:

"No male DNA profile was obtained from _____, (Item #)."

4. Statistics

4.1. The counting method is used to determine the frequency of a haplotype profile in a published database.

4.2. Single source samples and determined major haplotypes from mixtures will be searched in the U.S. Consolidated Y-STR Database online at http://www.usystrdatabase.org/

4.2.1. Follow the "User Directions" on the web page.

4.2.2. After the search is complete, the results (including the 95% Confidence Interval) shall be printed and included in the case file. The NCSBI will typically report the results for the searches against the African American, Caucasian, and Hispanic databases although other populations may be reported if necessary.

4.2.3. Partial profiles may be searched.

4.2.4. The upper bound confidence interval of 95% used by the U.S. Consolidated Y-STR online Database shall be observed and included in the report.

4.2.5. The result of the database search, including the number of matches and the 95% upper confidence intervals will be documented in the report. The results may be reported as follows:

"The male DNA profile developed from Item _ matches the YSTR profile developed from __ (Item _). Therefore, (name) and all his paternal relatives are not excluded as potential DNA donors to this sample. Utilizing a published YSTR DNA population database, this YSTR profile has been observed as follows:

_ in ___ Caucasians (Applying the 95% upper confidence interval results in approximately 1 in every ___ individuals.),

_ in __ African Americans (Applying the 95% upper confidence interval results in approximately 1 in every ___ individuals.), and

_ in ___ Hispanics (Applying the 95% upper confidence interval results in approximately 1 in every ___ individuals.)."

5. CODIS DATABASE

At this time, YSTR samples cannot be entered or searched in the CODIS database.

Revision History		
Effective Date	Revision Number	Reason
April 14, 2008	00	Original Document

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	